EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with David Clough on 1/12/2010.

The application has been amended as follows:

The title has been changed to the following:

Genome minimization by tn5-coupled cre/loxP excision system

In the specification:

At the paragraph beginning at page 11, line 21:

Figure ~~Figur3~~ 3A and 3B show the sites that can be inserted with TnKGloxP and TnCloxP in the E. coli genome.

At the paragraph beginning at page 16, line 2:

In the second step, the above synthesized double strand DNA was amplified in large scale by using transposon specific primer Tn5Int and primer Arb2 (5' - TTGAGCGATAGACGTACGAT-3' (SEQ ID NO: 12), synthesized by Genotech) whose base

sequence is identical to 25 sequence of 3' end of Arb1. The amplified DNA was separated from the agarose gel by using QIAQUICK spin PCR purification kit (Qiagen~~Quiagen~~), the base sequence of the above separated DNA was analyzed using primer Tn5Int (5' TCGACCTGCAGGCATGCAAGCTTCA-3' (SEQ ID NO: 13), synthesized by Genotech), and the insertion site was identified by comparing the above analysis result with GENBANK ~~GenBank~~ DNA sequence by using BLAST program. The identified insertion sites of TnKGloxP and TnCloxP by the above methods are shown in Figure 3.

In the claims:

5. (Currently amended) A method for constructing novel strains containing deletion of a specific chromosomal site, characterized in comprising the steps of:

(a) preparing a first transposon according to claim 1 ~~or claim 2~~ and a second transposon comprising the sequence of SEQ ID NO: 2~~according to claim~~ [[4]],

(b) inserting said first and second transposons, respectively, into random positions of different microbial chromosomes and determining the inserted sites;

(c) integrating the two microbial chromosomes by P1 phage transduction to position the first and second transposons on one chromosome; and

(d) deleting a chromosomal site between the two loxP sites by expressing Cre gene from a Cre expression vector.

6. (Canceled)

8. (Currently amended) The method for constructing novel strains according to claim 5 or claim 9, additionally comprising the steps of:

-selecting two mutants from the mutants containing deletion of a specific chromosomal site, and performing P1 phage transduction using one of the selected mutants as the donor and the other as recipient, to construct a new mutant containing all chromosomal deletion sites of the above two mutants; and

-using the above obtained mutant again as P1 phage recipient, and the already prepared mutant containing deletion of a specific chromosomal site as donor to perform P1 phage transduction continuously and repeatedly~~; and~~

~~-removing the chromosomal deletion site of other donor mutant from the chromosome of the obtained mutant continuously~~ to reduce the chromosome of the obtained mutant by degrees.

9. (New) A method for constructing novel strains containing deletion of a specific chromosomal site, characterized in comprising the steps of:

(a) preparing a first transposon according to claim 2 and a second transposon comprising the sequence of SEQ ID NO: 2,

(b) inserting said first and second transposons, respectively, into random positions of different microbial chromosomes and determining the inserted sites;

(c) integrating the two microbial chromosomes by P1 phage transduction to position the first and second transposons on one chromosome; and

(d) deleting a chromosomal site between the two loxP sites by expressing Cre gene from a Cre expression vector.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is (571)272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached on 571-272-0951. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Jennifer Dunston/
Examiner
Art Unit 1636